THE EMBRYOLOGY OF ALYSSUM.[1]

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(WITH PLATES XXVI–XXVIII)

THE following study of *Alyssum macrocarpum* was begun late in October when a large and thrifty plant was brought into the greenhouse. Cuttings were made which began blooming about the holidays and a constant supply of material was thus furnished.

My original intention was to study *Capsella bursa-pastoris* and to verify or modify the account of its embryonic development, since Capsella has usually been taken as a type for the dicotyls. While Hanstein's method of studying the embryo by squeezing it from the ovule and staining with iodine might appear incapable of yielding any results of a detailed character, it seems nevertheless in his hands to have given rather accurate results.

The close relationship of Alyssum to Capsella, and the resemblance in embryonic development, which was evident from the first comparison with Hanstein's familiar figures, became a constant stimulus to careful and accurate investigation. While I am not wholly positive as to the invariability in the formation of certain portions of the young embryo plant, the most of my work points to a certain definite course of development, though there are sometimes remarkable variations.

My sincere thanks are due to Professor W. A. Kellerman and Mr. J. H. Schaffner for their continued encouragement and valuable suggestions.

METHODS.

Greenhouse plants did not seed profusely, probably because there were no insects present to assist in pollination. Before killing the material, the sepals, petals and stamens were removed,

[1] Contributions from the Botanical Laboratory of Ohio State University. V.

except from very young buds, to insure rapid penetration. Chrom-acetic acid, and a solution of corrosive sublimate, acetic acid, and 70 per cent. alcohol were the two fluids used. For general purposes the first seemed preferable, though satisfactory results were obtained from both.

The material was imbedded in paraffin and cut into sections 12 μ thick. It was difficult to orient the ovaries so as to get sections parallel to the plane of the embryo sac, since the ovule of Alyssum is campylotropous and the embryo sac soon becomes curved like a horseshoe nearly parallel to the septum of the silicle.

Anilin-safranin, alone or in combination with gentian violet, and sometimes with a third, orange gentian, was used in staining. Acid fuchsin and iron-alum-hæmatoxylin were also employed. The latter was very useful in bringing out early stages of the embryo sac, but it was not so good after endosperm was present. Combinations with anilin-safranin were most satisfactory for general purposes.

A Bausch & Lomb microscope with $\frac{2}{3}$, $\frac{1}{8}$ and $\frac{1}{12}$ objectives, and 2 and 1 inch oculars, was used, and drawings were made with the aid of a Bausch & Lomb camera.

DEVELOPMENT OF MACROSPORES AND EMBRYO SAC.

The archesporial cell (*fig. 1*) is hypodermal in origin and can be recognized in the nucellus before the two outer integuments have entirely surrounded it. The nucleus of the archesporial cell is larger than those in other cells and the contents are more granular. By a transverse division a tapetal cell (*fig. 2, t*) is cut off, and this apparently undergoes no further division. The macrospore mother cell, however, divides into four cells, three potential macrospores (*fig. 2, p*) and a vital macrospore (*fig. 2, m*), which is the lowest of the series and develops into the embryo sac, destroying the entire nucellus in its growth (*figs. 2–12*).

The two-celled embryo sac (*fig. 3*) is nearly straight, with nuclei at opposite extremities and a well marked vacuole in the

center. The embryo sac increases in width in the four-celled stage (*figs. 4, 5*), and when it has reached the eight-celled stage it has a decided curvature (*fig. 6*). Many of the eight-celled embryo-sacs were destroyed or rendered worthless for camera drawings because they were cut and scattered through too many sections. The one figured has been sectioned so as to cut off the antipodals. It is probable that one is looking at them from above rather than in side view, as the rest of the figure appears. During the last divisions of the embryo sac the tapetal cell disappears (*figs. 5, 6*).

Up to this point the nuclei are uniform in size, but after the conjugation of the polar nuclei the definitive nucleus is easily distinguished by reason of its superior size, the presence of numerous refractive particles in its large nucleolus, and the readiness with which it stains. The presence of refractive bodies is not peculiar to the nucleolus of the definitive nucleus alone, but is found in less degree in the endosperm nuclei and in the large nucleus of the basal suspensor cell. It may also be true of other nuclei, but is not noticeable on account of the diminutive size.

After the definitive nucleus is formed, the egg apparatus (*figs. 7–9*) is readily distinguished, but in the majority of cases the antipodals have entirely disappeared. They are either absorbed or destroyed by the progress of the embryo sac in its encroachment upon the lower part of the nucellus, crushing and crumpling them out of shape. In many cases the lower part of the nucellus is contorted and pushed aside by the advance of the embryo sac, indicating a remarkable degree of force exerted from within (*fig. 12*). Where remains of antipodals were found, that end of the embryo sac had evidently slipped between the nucellus and its integuments and had not been subjected to the usual ordeal (*figs. 35–36*).

The oosphere is well concealed by the synergids (*figs. 8, 9*) until after fertilization, when its nucleus descends into the lower part and the oospore (*fig. 10*) begins to elongate rapidly (*figs. 11, 12*). Up to this time the definitive nucleus remains

undivided, but after fertilization takes place endosperm is formed very rapidly and is especially abundant around the proembryo, obscuring it and making it difficult to distinguish the early stages of embryonic development if the section was cut diagonally.

DEVELOPMENT OF THE EMBRYO.

The first division in the proembryo is transverse and cuts off a basal suspensor cell which does not divide again (*fig. 13, a*). The cell at the end divides very soon into a terminal cell, a true embryo cell (*figs. 13, 14, c*) and an intermediate cell (*figs. 13, 14, b*). The terminal embryo cell divides by a longitudinal wall into two cells (*fig. 15, c*), and the two following longitudinal divisions at right angles to the first cut it into quadrants (*fig. 16, c*). The third division is a series of transverse walls in the cells of the quadrant and produces an octant (*fig. 17, c*).

The dermatogen is the first tissue to be differentiated and is cut off from the octant by a series of periclinal and anticlinal walls (*figs. 18, 19*). Division in the cells of the octant is evidently almost simultaneous, for I could find no instance where any one seemed to have priority in the process. The terminal embryo cell is now represented by two well developed tiers of cells (*figs. 19, 20, c, d*). In each tier, while the dermatogen divides radially, the inner cells undergo a longitudinal division.

Meanwhile the intermediate cell (*fig. 14, b*) has been dividing by a series of transverse walls, and in the cell next those derived from the terminal embryo cell (*fig. 19, e*), the wall has become somewhat rounded. This cell divides, first into two and then into four cells by longitudinal walls, making a quadrant. These divisions and the transverse divisions which follow and form the octant correspond to those which take place in the terminal embryo cell, and, since this cell develops into the lower part of the embryo, I shall henceforth refer to it as the basal embryo cell. The whole embryo is therefore developed from two original cells, a terminal embryo cell and a basal embryo cell, both of which first form quadrants by longitudinal walls, and subsequently octants by a series of transverse walls in

each of the quadrants. *Fig. 27* represents a peculiar variation or abnormality in the development of the region of the basal embryo cell.

To the inner cells of the tier *d*, *fig. 20*, one can refer the origin of plerome and periblem, the central cells forming the plerome and the single layer between this and the dermatogen giving rise to the periblem.

The transverse division begun in the dermatogen has extended throughout this tier *d* (*fig. 21*), and is followed by another radial division in the dermatogen, while a longitudinal division occurs in the innermost cells of tier *c* (*fig. 24*). A series of longitudinal walls also begins to appear in tier *d*, followed by irregular transverse divisions (*fig. 25*). The entire embryo is now developing rapidly. The basal embryo cell has undergone the third and transverse division, forming two tiers of four cells each next the suspensor (*fig. 25*, *f* and *g*). In the region where the cotyledons arise, diagonal division has occurred, while in the region of the stem tip it has been longitudinal. The plerome is quite distinct and is shaded in the figure.

A more advanced stage is shown in *fig. 28*. The cotyledons develop more rapidly and the embryo becomes obcordate (*figs. 29–31*). The plate of cells nearest the suspensor undergoes a series of transverse divisions as well as longitudinal ones. This division extends to some of the adjoining dermatogen cells (*fig. 30*), and gives rise to the calyptrogen, which becomes continuous with the dermatogen and by successive transverse divisions cuts off the root cap. The inner plate, by longitudinal divisions, contributes to that part of the root tip from which the periblem of the radicle is developed (*fig. 33*, *f*, *g*). Beyond this stage it is impossible to get central sections through the entire embryo. The cotyledons fold together and curve upward toward the antipodal region (*fig. 34*), and in the mature state the embryo fills the entire cavity of the embryo sac.

THE SUSPENSOR.

The basal suspensor cell never divides after the first transverse division of the oospore. The intermediate cell contributes

toward the suspensor by a series of transverse divisions, which at first apparently occur in acropetal order (*fig. 15*), but later stages seem to indicate intercalary division (*figs. 22, 23*). The number of cells is variable, some large embryos having only eight- or nine-celled suspensors, while in others the number of cells reaches fifteen. In a few cases, instead of the normal transverse division in the suspensor cells, a longitudinal division occurs, giving a peculiar abnormal appearance (*figs. 22, 26,* and *27*).

The function of the suspensor seems to be that of an absorbing organ, supplying nourishment to the rapidly growing embryo and serving the purpose of the root in mature plants. When stained with anilin-safranin or its combinations, the suspensor and the cells arising from the basal embryo cell stained much less deeply than those derived from the terminal embryo cell. This was of great advantage in clearly determining the origin of calyptrogen, root cap, and root tip.

The suspensor persists until the embryo is mature, although it becomes shriveled and apparently functionless some time before the resting period of the embryo is attained.

ENDOSPERM.

The definitive nucleus divides immediately after the formation of the oospore. The endosperm accumulates very rapidly in the region of the proembryo and often obscures it, especially in the early stages. Early in my work I concluded that endosperm was formed previous to fertilization, because I frequently found it when I could not distinguish any embryo. But later research showed that endosperm was not present until after the proembryo appeared; and, whenever found, remains of a badly sectioned embryo were evident. Several instances in which unfertilized oospheres were found in the same silicle with well developed embryos showed the definitive nucleus distinct and undivided in the shriveled embryo sac, and an entire absence of endosperm.

The endosperm forms a complete lining for the embryo sac (*fig. 12*), and then, passing through the well-known radiations,

forms cell walls. Some of the free cells accumulate in the antipodal region and form a peculiar thallus-like mass, which further assists in obscuring whatever remains of the antipodals might otherwise be found (*figs. 35–39*). This growth was for some time a puzzle as to its origin, whether the result of division of the antipodals, or a growth of endosperm arising from the first division of the definitive nucleus and cut off by a cell wall from other endosperm, as is the case in Sagittaria.[2] All doubt was dispelled when the remains of the antipodals were found (*figs. 35, 36*) while this thallus-like growth was in early stages of development. In many cases the appearance was so peculiar and the connection with all other endosperm so obscured, while the entire mass stained so similarly to the embryo, that it might easily deceive one as to its nature. Its function is not clearly evident, unless it may be considered as a reserve of food material after the suspensor ceases to supply nourishment.

COMPARISON WITH OTHER DICOTYLS.

Chamberlain[3] says the archesporial cell in Salix divides into a tapetal cell which sometimes gives rise to a tier of five or six cells but occasionally does not divide; and a macrospore mother cell which may or may not divide. If it does there is a potential macrospore which sometimes divides and a vital macrospore which develops without further preliminary division into the embryo sac.

In *Aster Novae Angliae*[4] he reports that after the expected division resulting in four cells, the lowest usually develops into the embryo sac.

Coulter[5] says of *Ranunculus multifidus:* "In no case was a primary tapetal cell cut off, the archesporial cell dividing directly into mother cells." Alyssum might therefore be considered more primitive in this respect than any of these except Salix, which shows a very unsettled state of affairs.

In the development of the embryo of Alyssum there is much

[2] Schaffner, BOT. GAZ. **23**: 252–273. 1897.
[3] BOT. GAZ. **23**: 147–179. 1897.
[4] BOT. GAZ. **20**: 205–212. 1895.
[5] BOT. GAZ. **25**: 73–88. 1898.

more regularity than in that of any other dicotyledonous embryo yet studied. Capsella is undoubtedly quite symmetrical and Alyssum comes very close to it in many respects. I regret that it was not possible for me to make comparisons directly with Hanstein's text and figures, as there is so much variation in the illustrations and reprints given in text-books.

In Vines's *Text-book of Botany*, p. 443, the terminal embryo cell is figured as dividing first transversely, then longitudinally, while the text reverses this. In Goebel's *Outlines*, p. 397, the figures represent the first division as longitudinal, the second as transverse, while the text gives the first and second as longitudinal and the transverse divisions as a third series. This corresponds to Alyssum. In Sachs's *Text-book*, p. 516, 1875, the figure is the same as in Goebel's, but in the text the third series of divisions is given as tangential and cutting off the dermatogen from the quadrants. Whether this statement is due to faulty translation or is so in the original German edition, I cannot say. It does not agree, however, with the German edition of 1882, for there it is distinctly stated in the text that the first three series of divisions are in three directions at right angles to each other, although in the explanation of *fig. 446, I–IV*, he gives the second series of divisions as transverse, and does not figure or mention the second longitudinal division.

Confusion likewise exists with regard to that portion of the embryo of Capsella which Hanstein designates as the "hypophysis." Its origin is uncertain. It is thought to arise from the last division of the suspensor cell; *i. e.*, the cell which gives rise to the greater part of the embryo remains dormant after the first division in the proembryo, while the suspensor cell continues to divide. The last cell arising thus contributes to the embryo. Chamberlain[6] doubts the accuracy of this theory, but thinks it probable that the terminal cell in which the first longitudinal division appears is wholly embryonic, while there may be a varying number of cells in the suspensors formed before this division occurs. This agrees with Alyssum. Hanstein's

[6] BOT. GAZ. 23: 147–179. 1897.

"hypophysis," whatever its origin, has fallen into much the same confusion as the terminal embryo cell. While the majority of text-books give its first division as transverse, followed by longitudinal divisions, the figures and texts disagree as to the subsequent development of its tissue. In Goebel's *Outlines*, while the text describes the plate of cells, *h*, of *fig. 326*, as dividing to form calyptrogen and root cap, in the figure itself *h'* is divided instead of *h*. In Sach's *Text-book*, *h'* is so figured and the text corresponds.

It is unfortunate that such confusion exists in the embryology of Capsella, and however careful and accurate Hanstein's original work may have been, he is either ambiguous in his statements or he has been mistranslated. It seems quite probable that Capsella is very close to Alyssum in its embryonic development and the many resemblances existing between some of the stages shown in my drawings and those of Hanstein seem to lead one to the conclusion that their embryology is very similar. Perhaps a re-investigation of Capsella would show its development to be the same.

SUMMARY.

1. The hypodermal archesporial cell divides into a tapetal cell and a macrospore mother cell which gives rise to four macrospores, three potential macrospores and a vital macrospore, the lowest of the series, which develops into the embryo sac.

2. The embryo sac passes through the usual cell divisions, increasing in size and becoming much curved, until the entire nucellus is destroyed.

3. The antipodals are ephemeral, disappearing during the early stages of embryonic development.

4. The endosperm appears soon after the fertilization of the oosphere.

5. The first division of the proembryo is transverse and the basal suspensor cell never divides afterward.

6. The end cell divides into the intermediate cell which contributes both to suspensor and embryo, and a terminal embryo cell.

7. The first and second series of divisions in the terminal embryo cell are longitudinal and form a quadrant. The octant is formed by a series of transverse divisions.

8. The first three series of divisions in the basal embryo cell correspond to those of the terminal embryo cell, also giving rise to an octant.

9. The dermatogen is the first tissue which is differentiated and is cut off by tangential walls in the cells of the octants.

10. The plerome and periblem arise in the basal hemisphere of the terminal embryo cells; the cotyledons and stem tip from the terminal hemisphere.

11. The calyptrogen and root cap are formed from the basal hemisphere of the basal suspensor cell, while that part of the root tip which forms the periblem of the radicle arises from the hemisphere lying next to the terminal embryo cells.

12. The number of cells in the suspensor varies from six to fifteen. The number beyond six apparently depends upon the number of intercalary divisions, some of which may be longitudinal.

13. The endosperm lines the entire embryo sac with a single layer of cells, but is more abundant around the young embryo and forms a peculiar growth in the antipodal region.

COLUMBUS, OHIO.

EXPLANATION OF PLATES XXVI–XXVIII.

Drawings reduced to three-eighths; the magnification given with each figure refers to the original magnification before reduction.

PLATE XXVI.

FIG. 1. Nucellus and integuments; archesporial cells. ×1060.

FIG. 2. Nucellus with tapetal cell, *t*; potential macrospores, *p*; and vital macrospore, *m*. ×1060.

FIG. 3. Two-celled embryo sac; tapetal cell, *t*. ×1060.

FIGS. 4–5. Four-celled embryo sac. ×1060.

FIG. 6. Eight-celled embryo sac. ×1060.

FIG. 7. Seven-celled embryo sac. ×1060.

FIG. 8. Egg apparatus and definitive nucleus. ×580.

FIGS. 9 and 9a. Embryo sac before fertilization; antipodals, *a*. ×1060.

FIG. 10. Embryo sac after fertilization; oospore, *o*; synergids, *s*. ×1060.

FIG. 11. One-celled proembryo; pollen tube, *p t*; synergid, *s*; a peculiar endosperm nucleus, *end. nu.* ×1060.

FIG. 12. Embyo sac showing relative size of proembryo and the distribution of endosperm. ×1060.

PLATE XXVII.

FIG. 13. Three-celled proembryo; basal suspensor cell, *a*; intermediate cell, *b*; terminal embryo cell, *c*. ×1060.

FIG. 14. Same, farther advanced. ×1060.

FIG. 15. First longitudinal division of the terminal embryo cell, *c*. ×1060.

FIG. 16. Second longitudinal division of *c*. ×1060.

FIG. 17. Octant stage, two nuclei cut away in sectioning; intermediate cell, *b* much divided. ×580.

FIG. 18. Embryo with dermatogen. ×580.

FIG. 19. Same, with more cells in suspensor. ×580.

FIG. 20. Embryo showing remains of pollen tube, *p t*; basal embryo cell, *e*; the terminal embryo cell is divided into two distinct tiers, *c* and *d*. ×580.

FIG. 21. Embryo showing a series of transverse divisions in tier *d*, and two longitudinal divisions in basal embryo cell, *e*. ×580.

FIG. 22. Suspensor which seems to indicate intercalary division. ×1060.

FIG. 23. Abnormal suspensor in which intercalary division has been longitudinal instead of transverse; plerome shaded. ×1060.

FIG. 24. More advanced embryo showing longitudinal division in plerome. ×1060.

FIG. 25. Transverse division following the longitudinal; basal embryo cell divided into two tiers, *f* and *g*. ×580.

FIGS. 26 and 27. Embryos from same silicle, showing abnormal suspensor and basal embryo cell. Shaded and light areas show difference in staining where triple staining was used. ×1060.

FIG. 28. Embryo having four tiers in plerome and two in the region where the stem tip and cotyledons originate. ×580.

PLATE XXVIII.

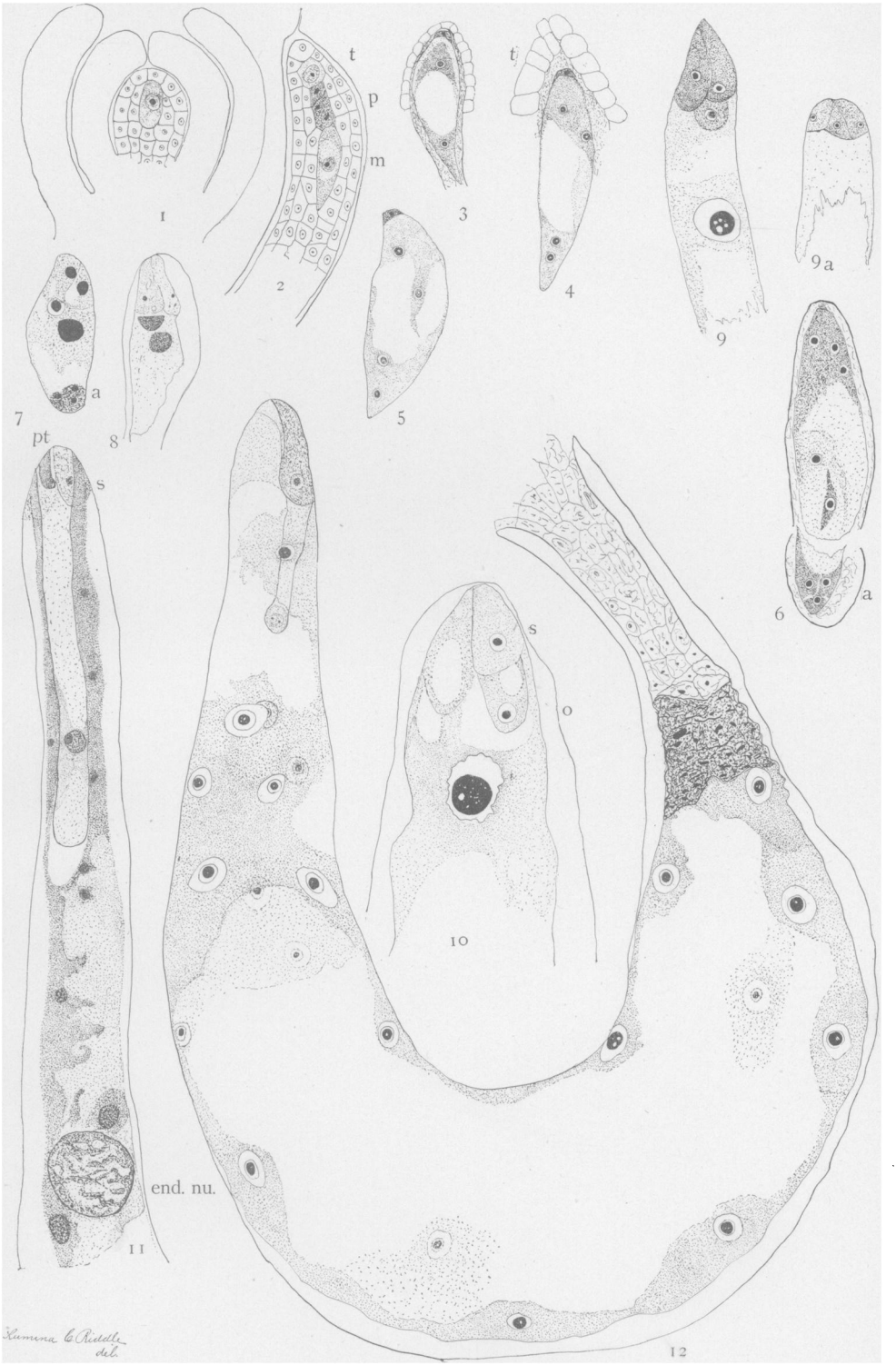
FIG. 29. Embryo with four tiers in plerome; a series of longitudinal walls appearing in the inner tier; three rows in region of cotyledons. ×1060.

FIGS. 30–32. Large embryos showing development of calyptrogen, root cap and root tip. ×580.

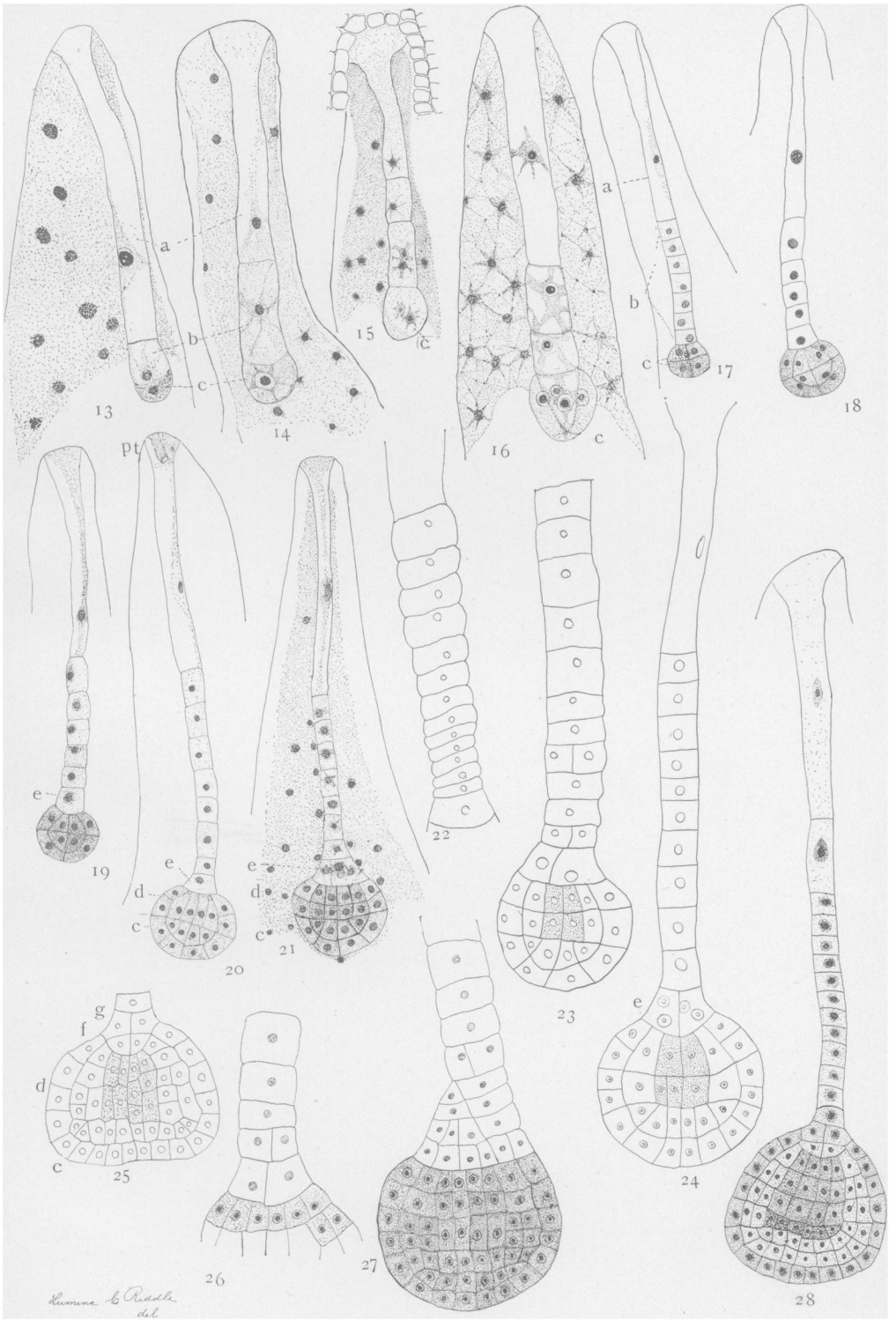
FIG. 33. Section through root tip, showing plerome, periblem, dermatogen, calyptrogen, and root cap. ×590.

FIG. 34. Nearly mature embryo showing suspensor still persisting. ×90.

FIGS. 35–39. Antipodal end of embryo sac, showing antipodals disappearing and endosperm forming thallus-like growth. *Figs. 35–38*, × 1060; *fig. 39*. × 590.



RIDDLE on ALYSSUM



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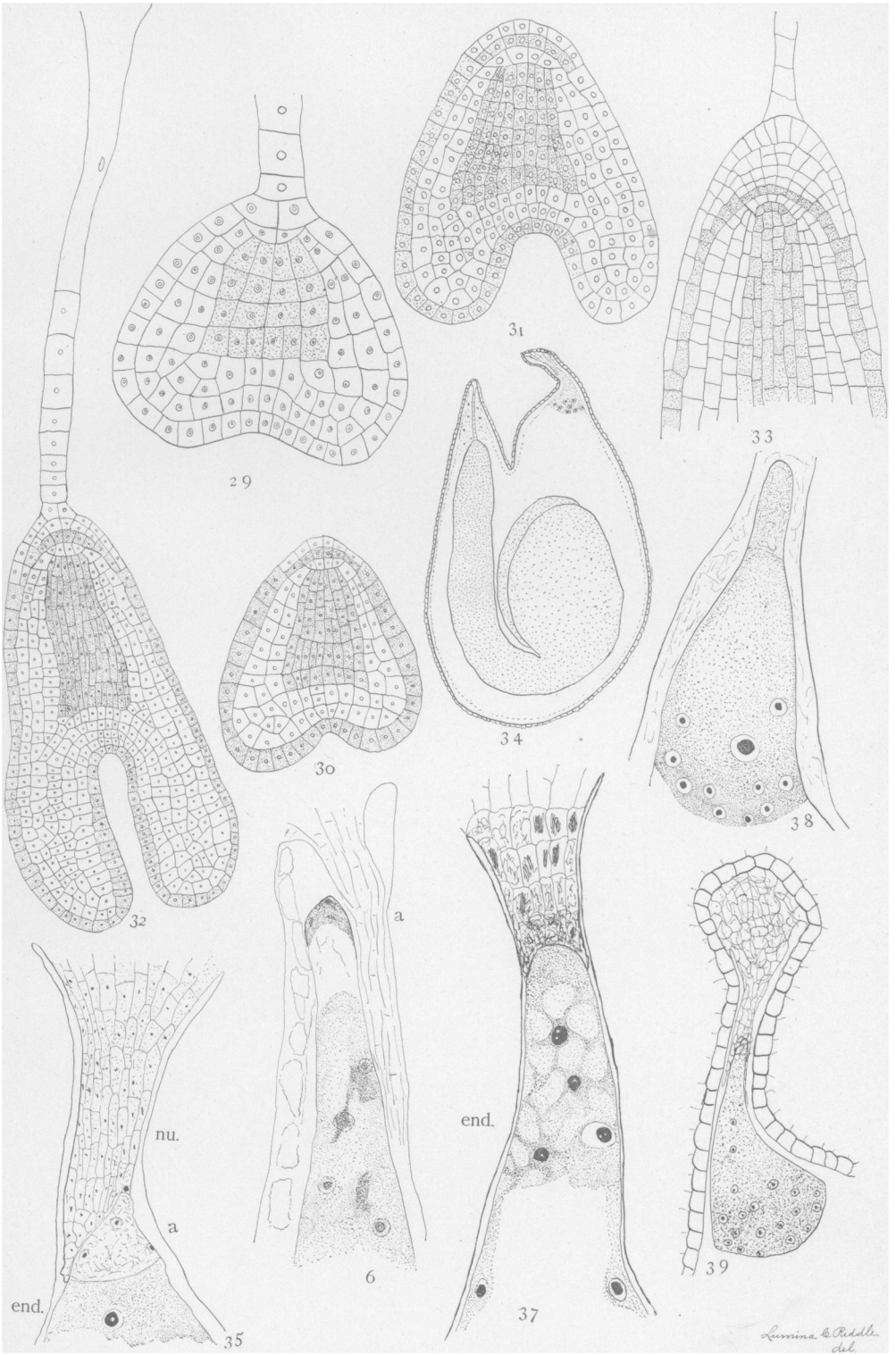

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